Discovery and the genetic basis of a thermo-sensitive genic ms line with large flowers in *Brasscia napus* L.

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Abstract

104S is a new TG ms line which has been found and bred by means of systematic breeding from the plant group of inbreeding 99104 in *Brassica napus*. The investigations of fertility show that 104S has the similar changing regularity of fertility to the previous TG ms lines such as Xiangyou 402S, but is different in flower morphology from the latter. The petals of 104S are larger obviously than those of Xiangyou 402S. The genetic studies indicate that the fertility of 104S is controlled jointly by a couple of recessive ms genes and a couple of mainly effective thermo-sensitive genes with some minor genes, and its ms genes and thermo-sensitive genes are not the allele of that of Xiangyou 402S.

Key words: *Brassica napus* L, thermo-sensitive genic ms, breeding; flower morphology, genetic basis

Thermo-sensitive genic ms (TG ms) line is a new type of ms line in *Brassica napus*, which is utilized through reproducing itself when it is in the state of fertility and producing F_1 hybrid when it is in the state of sterility grown in different seasons or in different ecological regions. The fertility of TG ms line is controlled by recessive ms genes and thermo-sensitive genes together, which means that the restorers are easily found and the fine combinations with strong heterosis can be easily gained.

The first TG material P_{3-3} of rape was discovered in 1991, and a stable ms line Xiangyou91S [1] was successfully bred by means of systematic breeding from P_{3-3}. Using Xiangyou 91S, some fine new TG ms lines such as Xiangyou 402S [2] have been bred. One remarkable character of Xiangyou 91S and Xiangyou 402S is that their fertility changes with the temperature changing during the forming stage of their flowers. The lines display complete male-fertility when the temperature is lower than one critical low point, and display complete male-sterility when the temperature is higher than one critical high point, and display partial male-sterility when the temperature is between the low critical point and the high critical point [3]. With the fertility changing, the morphology of flowers changes too. Their petals and stamens become smaller and smaller with the sterile degree increasing, but the other parts of flower do not change apparently.

A new thermo-sensitive ms material with large flowers was found from the plant group of a restorer of TG ms line in the procedure of seed production of TG ms two-line hybrid. Some studies on the fertility, flower morphology and genetics of this ms material have been conducted, which are reported as follows.

1 Materials and Methods

1.1 Materials

Inbreeding line 99104 with double-low (low erucic acid and low glucosinolate) quality and fine agronomic traits which was bred from the offspring of multi-crossing {[(Zhongyou821×84039)×325] ×325} used as the restorer of TG ms line, two test-crossing lines : 04014 and 04107, TG ms line Xiangyou402S, recessive genic (RG) ms line and its maintainer Xiangyou402AB, are all from Crops Research Institute of Hunan Academy of Agricultural Sciences.

1.2 Methods

1.2.1 Discovery and breeding of 104S

One thermo-sensitive ms plant was found among the plants of 99104 after their stems were cut down in the procedure of hybrid seed production of the combination (Xiangyou 402S×99104) in spring 2000. This plant displayed normal fertility before it was stemmed and displayed partial sterility or complete sterility after being stemmed.

The ms plant was reproduced through inbreeding using those partially sterile flowers. Systematical selection had been carried out in the inbreeding offspring according to the agronomic traits, quality traits and resistance to *Sclerotinia sclerotiorum* and virus. A stable ms line with the similar agronomic traits to 99104 and double-low quality from the offspring of this ms plant was gained in 2004, which was named as 104 S. Among the inbreeding offspring from the same ms plant, one completely male sterile plant was also found in 2001, which was crossed with one plant of 99104. From this combination, one stable group with 50% fertile plants and 50% completely ms plants had been gained in 2004, which was named as 104AB.

1.2.2 Investigation of the fertility of different ms lines

Investigations for the fertility of the following materials: 104S, 104A, 402S, 402A and 99104, were made by means of continual observation in 2004. Every material was planted in the plot with area of 6.67 m^2. Five typical plants of every

material were selected out for the fertility investigation. The investigation was carried out once five days from the beginning of flowering, and the flowers were taken off after investigation. The petals from ten flowers of every material were measured at five main stages. The fertility was identified according to the methods in reference [4]. Average fertility index (AFI) is used as the fertility data of plant group. AFI=(1×flower number of grade one+2×flower number of grade two+3×flower number of grade three+4×flower number of grade four+5×flower number of grade five+6×flower number of grade six)/total number of all flowers. The fertility index of plant group is bigger, the sterility degree is lower. It is defined here that the plants with AFI more than 5.0 are fertile (F), and the plants with AFI from 1.0 to 5.0 are partially sterile (PS) and the plant with AFI less than 1.0 are completely sterile (CS).

1.2.3 Genetic studies

The following crossings were made to get F_1 generation in 2004: 104S×04014, 104S×04107, 04014×104S, 04107×104S, Xiangyou402A×104S, 104A×Xiangyou402S. The F_1 generation of every combination was planted in Autumn of 2004 and the inbreeding was made in Spring of 2005 to obtain F_2 generation. The F_2 generations were planted in Autumn of 2005, and the fertility of F_2 was investigated in Spring of 2006. In the process of fertility investigation, the thermo-sensitive plants with different transforming stage were noted respectively, and classified as three groups: turning early, turning late and the middle type between the early and the late.

2 Results

2.1 The fertility and morphological characters of flowers of different ms lines

The results of fertility investigation (Table 1) show that Xiangyou 402A and 104A are completely sterile (The fertility index of both lines are 0) and 99104 is fertile (The fertility index is more than 5.0) in total flowering stages, and the fertility of Xiangyou 402S and 104S changes (The fertility index changes from 0 to 5.17 and from 0 to 5.54 respectively) with the developing process. Before 10 March, Xiangyou 402S and 104S are all fertile. From 10 to 20 March, some flowers of Xiangyou 402S turn to partial sterility, while all flowers of 104S are still fertile. From 20 to 30 March, most flowers of Xiangyou 402S still display partial sterility, and some flowers of 104S begin to transform to partial sterility but most flowers are still fertile. From 30 March to 9 April, most flowers of Xiangyou 402S turn to complete sterility and only a small amount of flowers still keep partial sterility. At this stage, most flowers of 104S are partially sterile and only a little of flowers had transformed to complete sterility. After 9 April, all flowers of Xiangyou 402S are completely sterile, while the flowers of 104S do not turn to complete sterility until 19 April.

With regard to the fertility change, it can be found that 104S has the similar regular to Xiangyou402S of which flowers transform from fertility to partial sterility then to complete sterility with the growing process. Nevertheless, 104S transforms from fertility to sterility later than Xiangyou402S does.

Table 1 The fertility of different material at different stage (Changsha, 2004)

Material	Average fertility index											
	03-05	03-10	03-15	03-20	03-25	03-30	04-04	04-09	04-14	04-19	04-24	04-29
XY402S	5.17	5.01	4.26	4.14	3.85	2.63	1.26	0	0	0	0	0
XY402A	0	0	0	0	0	0	0	0	0	0	0	0
104S	5.54	5.47	5.62	5.25	4.47	3.82	2.96	2.55	1.61	0	0	0
104A	0	0	0	0	0	0	0	0	0	0	0	0
99104	5.74	5.81	5.48	5.44	5.37	5.17	5.33	5.42	5.51	5.36	5.16	5.25

XY402S and XY402A are the abbreviation of Xiangyou402S and Xiangyou402A respectively.

With the fertility changing, the flower morphology of Xiangyou 402S and 104S change too. Their pistils, sepals and honey-body do not change obviously. The stamens of both ms lines diminish with the sterile degree increasing until they become small trigon without pollen. The petals of Xiangyou 402S change very greatly in size, while the petals of 104S do not change apparently. From Table 2, it can be found the petals of 104A are apparently bigger than that of Xiangyou402A, and the petals of Xiangyou402S and 104S are almost as big as that of 99104 when they are completely fertile before 12 March, and almost as big as that of Xiangyou402A and 104A respectively when they are completely sterile. The average size of 104S (9.14×14.74 mm×mm) is apparently bigger than that of Xiangyou402S (7.82×13.16mm×mm).

Table 2 The average size of petals of different material at different growing stage

Material	Average width and length of petal / mm×mm					Average value/mm×mm
	03-10	03-20	03-30	04-09	04-19	
XY402S	10.4×14.8	10.7×15.1	8.1×14.4	6.8×11.5	6.1×10.3	8.42×13.22
XY402A	7.4×11.5	6.9×12.1	7.2×11.4	6.5×11.7	6.5×10.8	6.90×11.50
104S	11.1×15.4	11.5×15.5	10.1×14.7	8.6×14.3	8.3×14.1	9.92×14.80
104A	9.5×15.2	10.1×15.7	9.8×15.0	9.3×14.7	9.1×14.4	9.56×15.0
99104	10.6×15.1	11.1×15.4	11.3×15.7	11.5×15.4	10.8×14.9	11.06×15.30

2.2 Genetic characters of 104S

The investigating results (Table 3) for the fertility of F_1 and F_2 generation of the combinations of 104S with two test-crossing lines in both modes of positive and negative crossing show that all plants of F_1 generations are fertile, and the plants of F_2 generation of four combinations all separate in the proportion of about 3 (fertile ones) : 1(completely and partially sterile ones), which mean that the fertility of 104S is jointly controlled by a couple of recessive nuclear genes and thermo-sensitive genes, and the cytoplasm has no effect.

Table 3 The fertility of plants of F_1 and F_2 generations of the combinations with 104S in positive and negative crossing mode

Combination	F_1 generation		F_2 generation					χ^2	$\chi_{0.05,1}^2$
	Fertile plants	Sterile plants	Fertile plants	TS plants	CS plants	The fertile/ the sterile*	Expected proportion		
104S×04014	127	0	1772	406	149	1772:555	3:1	1.5793	3.84
104S×04107	114	0	1841	438	173	1841:611	3:1	0.0049	3.84
04014×104S	121	0	1514	357	141	1514:498	3:1	0.0537	3.84
04107×104S	126	0	1702	443	158	1702:591	3:1	0.6921	3.84

*Notes: The sterile plants contain partially and completely sterile plants.

Meanwhile, the proportions of thermo-sensitive plants to completely sterile ones in F_2 generations of four combinations all accord with 3:1, and there are three kinds of thermo-sensitive plants with different transforming stage, which show that the thermo-sensitive genes of 104S contain a couple of mainly effective genes and some minim genes (Table 4).

Table 4 The distributions of plants with different sterile degree in F_2 generations

Combination	TS plants			CS plants	The TS: the CS	Expected proportion	χ^2	$\chi_{0.05,1}^2$
	turning early	middle type	turning late					
104S×04014	35	274	107	149	406:149	3:1	0.9135	3.84
104S×04107	84	229	125	173	438:173	3:1	3.4048	3.84
04014×104S	88	176	93	121	357:141	3:1	2.7416	3.84
04107×104S	61	306	76	158	443:158	3:1	0.4743	3.84

2.3 The allelism of different ms genes and thermo-sensitive genes

Among the plants of F_1 generations of combination Xiangyou402A×104S and 104A×Xiangyou402S, sterile or TS plants had not been found (Table 5), which means that the ms genes in Xiangyou402S and 104S are not allele.

In the offspring of F_2 generations of two combinations, fertile, TS and completely sterile plants were found. Two kinds of plants with different size of flowers were found among the completely sterile plants of both combinations respectively. One kind had big flowers and another had small flowers. However, only plants with big flowers were found among the TS plants of combination Xiangyou402A×104S, and only ones with small flowers were found among the TS plants of combination 104A×Xiangyou402S, which means that one type of thermo-sensitive genes can just only control the corresponding ms genes, but can not effect another ms genes. Therefore, the thermo-sensitive genes of Xiangyou402S and 104S are not allele too.

The proportions of plants with different fertility in the F_2 generation of two combinations show that Xiangyou402S has two couples of recessive ms genes and a couple of thermo-sensitive genes, and 104S has a couple of recessive ms genes and a couple of thermo-sensitive genes.

Table 5 The fertility of offspring of the combinations concerning four ms lines

Combination	F_1 generation								F_2 generation			
	Fertile plants	Sterile plants	Fertile plants	TS plants	CS plants	The fertile:the sterile	Expected proportion	χ^2	The TS:the CS	Expected proportion	χ^2	$\chi_{0.05,1}^2$
XY402A×104S	109	0	1627	413	261	1627:674	180:76	0.1543	413:261	45:31	1.1064	3.84
104A×XY402S	115	0	1754	94	637	1754:731	180:76	0.0748	94:637	9:67	0.6293	3.84

3 Summary and Discussion

According to above results, 104S is a new TG ms line different from Xiangyou402S in flower morphology and genetic base.

104S has the similar changing regular of fertility with the temperature changing at the flowering stage to Xiangyou402S, in which the sterile degree will increase till complete sterility with temperature increasing, but the petals of 104S do not change obviously unlike Xiangyou402S. The fertility of 104S is jointly controlled by a couple of recessive ms genes and thermo-sensitive genes which contain a couple of mainly effective genes and some minim quantitative genes.

From the fertility investigation of 104S, it is found that the transforming stage of 104S is later than that of Xiangyou402S, which means that 104S has a higher critical temperature from fertility to sterility than Xiangyou402S. This character of 104S is not beneficial to the utilization, because a high critical temperature may lead to trace pollens which influence the purity of hybrid seed in the process of seed production. Therefore, it is necessary to improve 104S to breed new ms lines with proper critical temperature of fertility transforming. From the F_2 generations of positive and negative combinations of 104S with two test-crossing lines, various thermo-sensitive plants with different transforming stage were found, which means that it is

feasible to breed fine ms lines with proper critical temperature.

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